Journal of Surgical Oncology 63:91–94 (1996)

DNA Index by Image Analysis in Advanced Endometrial Carcinoma

JOHN P. GEISLER, MD, MICHAEL C. WIEMANN, MD, ZHEN ZHOU, PhD, GREG A. MILLER, MD, AND HANS E. GEISLER, MD

From the Division of Gynecologic Oncology, Department of Obstetrics and Gynecology (J.P.G., H.E.G.), Division of Oncology, Department of Medicine (M.C.W.), St. Vincent Hospital and Health Care Center, Indianapolis, Indiana; Department of Pathology, Laboratory for Diagnostic and Analytical Cytometry (Z.Z., G.A.M.), Indianapolis, Indiana

Background: Endometrial carcinoma is the most common gynecologic malignancy in developed countries, affecting an estimated 140,000 women. More than 32,000 women will be diagnosed with endometrial cancer this year in the United States, and approximately 6,000 will die from this disease.

Methods: Twenty consecutive patients, surgically treated, with advanced endometrial cancer, were evaluated for their DNA index (DI), time to recurrence, peritoneal cytology, depth of invasion, lymphovascular space invasion, as well as FIGO stage, grade, and histology. DI was determined using image analysis.

Results: Ten of the 20 patients had recurrence of their disease within the 3-year observation period of the study. A DI of ≥ 1.2 strongly predicted recurrence of disease ($P = 0.002$). Increasing histologic grade and an increasing DI were related ($P = 0.01$).

Conclusion: Independent of other prognostic indicators, including lymphovascular space invasion, depth of invasion, and histologic type, a tumor with a DI of ≥ 1.2, had a significantly increased chance of recurring within the 3-year observation period.

KEY WORDS: DNA index, recurrence, endometrial carcinoma

INTRODUCTION

Endometrial carcinoma is the most common gynecologic malignancy in developed countries and affects an estimated 140,000 women worldwide [1,2]. In the United States, in 1995, an estimated 32,800 women will be diagnosed as having endometrial cancer, while almost 6,000 will die from the disease [1]. Histologic type, grade, stage, depth of myometrial invasion, and lymphovascular space invasion are all recognized prognostic indicators in patients with cancer of the endometrium [3–6]. Over the past decade, the importance of DNA index (DI) and ploidy in endometrial carcinoma has become apparent [6–14].

DNA image analysis relies on an integrated, interactive computer system which measures specific cellular features [15]. It differs from conventional DNA flow cytometry by virtue of the fact that image analysis is a static process not necessitating specimen destruction. In image analysis, each cell to be analyzed is able to be individually viewed. Thus, nonmalignant cells can be readily eliminated from the specimen to be analyzed. Unlike conventional light microscopic criteria such as histologic grade, measurements from image analysis, including DI, are objective. The purpose of this study was to ascertain whether the DI, as determined by image analysis, was associated with other prognostic indicators, as well as recurrence, in patients with FIGO stage III and IV endometrial carcinoma.

MATERIALS and METHODS

Twenty consecutive patients with stage III or IV endometrial carcinoma, who had sufficient tumor mass re-

Accepted for publication June 25, 1996.

Address reprint requests to Hans E. Geisler, M.D., Department of Gynecologic Oncology, St. Vincent Hospital and Health Care Center, 8424 Naab Road, Suite 2M, Indianapolis, IN 46260.

maining after steroid receptor assays were performed, had their tumor analyzed for the DI. The DI was determined by image analysis. Patients' records were analyzed for age, FIGO stage, grade, histologic type, lymphovascular space invasion, peritoneal cytology, and time to recurrence. These observations were collected over a 3 year period. Primary treatment consisted of total abdominal hysterectomy, bilateral salpingo-oophorectomy, and selective bilateral pelvic and lower para-aortic lymph node sampling when appropriate. Additional treatment consisted of 5,000 cGy whole pelvis external beam radiation and vaginal cesium implants for all patients in this study. One hundred-sixty mg/day of megestrol acetate was added to the treatment of all patients in this study. Comparisons of the data were made with the SPSS statistical package, using bivariate analysis, multivariate analysis, or Student's *t*-test, where appropriate.

Specimen Preparation

Touch preparations for image analysis were prepared at surgery and air dried. Fresh or frozen tissue was submitted for cytometric evaluation. Fresh tissue was received in RPMI (Sigma, St. Louis, MO). Frozen tissue was stored at −70°C and thawed at analysis. Frozen and fresh tissue were prepared identically. Nuclei were mechanically disaggregated. The samples were passed through needles of increasing gauge (of 18–25) and then filtered through 53- and 33-μm nylon mesh (Small Parts, Miami, FL). After a cell count was done, each suspension was separated into aliquots for flow cytometry, image analysis, and morphologic evaluation. For image analysis, an aliquot of the nuclear suspension from each sample was cytocentrifuged onto a poly-L-lysine-coated slide (Sigma). The slides were air dried for 30 min, fixed in neutral buffered 10% formalin for 30 min, rinsed in three changes of deionized water (5 min each), and again air dried. Slides prepared at surgery, along with slides prepared from the flow cytometry sample, were then processed according to the procedure for Feulgen staining included in the Cell Analysis Systems (CAS) DNA staining kit (Cell Analysis Systems, Elmhurst, IL). The slides were placed in 5 N HCl for 60 min, stained with the Feulgen dye solution for 50 min, rinsed three times with the rinse solution, decolorized in acid alcohol for 5 min to remove any background stain, dehydrated, and coverslipped with Permout (Fisher Scientific, Itasca, IL). A calibration slide (CAS) containing rat hepatocytes was included with each staining run.

Image analysis was performed using a CAS-200 video image analyzer (CAS). Before data collection, the instrument was calibrated using a control slide of rat hepatocytes stained with each batch of slides. For each sample, a total nuclear optical density of at least 200 structurally well-preserved and separate neoplastic nuclei was obtained at a wavelength of 546 μm. The DNA content was derived from the total nuclear optical density and expressed as picograms of DNA. The internal diploid controls consisted of a mixture of nuclei from normal stromal cells and lymphocytes. For each sample, a DNA histogram was then generated and the DI of all main peaks determined. DI was calculated from the mean DNA content of a neoplastic G_0/G_1 peak.

A sample was considered diploid (DI = 1.0 ± 0.1) when a single G_0/G_1 peak occupied the same histogram position as the diploid control G_0/G_1 peak and no other G_0/G_1 peak with >10% of the total number of nuclei was present. An aneuploid population (DI not equal to 1.0) was defined as the presence of one or more G_0/G_1 peaks outside the diploid range and contained >10% of the total number of nuclei. The aneuploid samples were subclassified according to the value of DI (hyperdiploid, $1.1 < DI < 1.8$; tetraploid, $1.8 \leq DI \leq 2.2$; hypertetraploid, $DI > 2.2$; and multiploid, two DI ± 1.0). Besides having a DI of 1.8–2.2, tetraploid samples had to contain >20% of the total nuclei in the 2n G_2/M peak region on the histogram and nuclei in the 8n position (2n G_2/M peak for a tetraploid population). The DNA histograms that contained two closely overlapping peaks were classified as having questionable DNA ploidy.

RESULTS

Twenty patients with stage III or IV disease were identified. Eighteen of the 20 patients had stage III disease, while two had stage IV disease. Fourteen patients had endometrioid adenocarcinomas, two patients adenosquamous carcinomas, two papillary serous carcinomas, and two undifferentiated carcinomas.

The mean DI of the endometrioid adenocarcinomas (1.14) was significantly less than the DI of the others (1.51) ($P < 0.001$) by Student's *t*-test. Among the 20 patients with either stage III or IV disease, increasing histologic grade correlated with an increased DI ($P = 0.043$) by bivariate analysis. The mean DI index was 1.26, with a range of 1.00–2.54. The mean age was 69, with a range of 63–73 years. Bivariate analysis did not show a correlation between age and DI ($P = 0.49$). There was also no statistically significant difference in the mean age (68 years) of patients whose disease recurred, versus patients whose disease did not recur (70 years, $P = 0.80$).

Lymphovascular space invasion occurred in 6 of the 20 patients. The mean DI of the tumors in whom no lymphovascular space invasion occurred was 1.05, while in patients whose tumors showed lymphovascular space invasion, it was 1.75 ($P < 0.001$). Positive peritoneal cytology was found in 12 of the patients, including 6 of 12 patients with positive nodes. The mean DI of the tumors in patients with negative peritoneal cytology was 1.06, while in patients with positive peritoneal cytology the mean DI was 1.55 ($P = 0.001$).

TABLE I. Multivariate Analysis of Prognostic Factors in Advanced Endometrial Cancer

Variable	P value
Peritoneal cytology	0.90
Depth of myometrial invasion	0.16
Histologic grade	0.045
Histologic type (endometriod vs nonendometriod)	0.005
Lymphovascular space invasion	0.84
Lymph node status	0.16
DNA index ≥1.2	<0.001

TABLE II. Univariate Analysis of Prognostic Factors and Recurrence in Advanced Endometrial Cancer

Variable	P value
Peritoneal cytology	0.90
Depth of myometrial invasion	0.40
Histologic grade	0.90
Histologic type (endometriod vs nonendometriod)	0.36
Lymphovascular space invasion	0.36
Lymph node status	0.054
DNA index ≥1.2	0.0017

Retroperitoneal lymph nodes were positive in 12 of the 18 patients undergoing lymph node sampling. The mean DI of the tumors in patients with negative retroperitoneal lymph nodes was 1.42, while in patients with positive lymph nodes the mean DI was 1.15 ($P = 0.001$). In the four patients in whom only positive pelvic lymph nodes were found, the tumors were diploid, while in the eight patients in whom positive pelvic and para-aortic lymph nodes were found the tumors had a mean DI of 1.22 ($P = 0.044$). All patients in this study who had positive para-aortic nodes also had positive pelvic nodes.

Ten of the 20 patients had recurrence of their disease within the 3-year observation period of this study. The mean DI for the malignancies that did not recur within 2 years was 1.06, while the mean DI for the tumors recurring was 1.45 ($P = 0.004$) by Student's *t*-test. Increasing DI correlated with an increased chance of recurrence ($P = 0.043$). Multivariate analysis, using logistic regression, revealed that a DI of ≥1.2 was associated with a significantly increased chance of recurrence ($P < 0.001$) (Table I). Univariate analysis showed that a DI of ≥1.2 was also important in predicting recurrence (Table II).

DISCUSSION

A large number of pathologic and clinical factors have become established as prognostic indicators in patients with carcinoma of the endometrium, including FIGO stage, grade, histology, depth of myometrial invasion, and lymphovascular space invasion [3–6,16–19]. Although the authors believe that peritoneal cytology is important, it remains somewhat controversial [4, 20, 21]. DNA aneuploidy has been linked to overall decreased survival, but a similar link to a decreased disease-free interval has not been as successfully made [7].

Although there were a limited number of patients in this study, a link could be established between an increasing DI and recurrence of disease ($P = 0.043$). A DI of ≥1.2, with diploid 1.0, was associated with a significantly increased chance of recurrence, regardless of lymphovascular space invasion, histologic type, or depth of myometrial involvement ($P < 0.001$). These findings support those of Newbury et. al. [10] that aneuploidy correlates with decreased overall survival.

When analyzing the data, the fact that tumors with positive retroperitoneal nodes and no abdominal extension had a decreased mean DI, as compared to tumors with intraperitoneal spread, becomes prominent. Although the cohort size is small in this preliminary study, one wonders whether there is a difference in the inherent biologic activity of tumors that spread primarily through the lymphatics versus tumors that have a primary intraperitoneal route of spread. Another possible reason for the difference may be that the number of patients was too small. Therefore, an analysis of a larger group of patients is under way to prove or disprove this point.

Two important prognostic indicators in endometrial carcinoma—positive peritoneal cytology and the presence of lymphovascular space invasion—were shown to be associated with an increased DI. Tumors that recurred during the observation period of this study were shown to have an increased DI, further supporting the importance of DI as a prognostic indicator and helping to validate the use of image analysis in studying endometrial cancer.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the support of the John Marten Family Memorial Cancer Research Fund and Rod Fasone Memorial Cancer Research Fund.

REFERENCES

1. Wingo PA, Tong T, Bolden S: Cancer statistics, 1995. CA Cancer J Clin 45:8–30, 1995.
2. Boffetta P, Parkin DM. Cancer in developing countries. CA Cancer J Clin 44:81–90, 1994.
3. DiSaia PJ, Creasman WT: Adenocarcinoma of the uterus. In "Clinical Gynecologic Oncology" 4th Ed. Mosby-Year Book, St. Louis, 1993, p156–193.
4. Creasman WT, Rutledge F. The prognostic value of peritoneal cytology in gynecologic malignant disease. Am J Obstet Gynecol 110:773–781, 1971.
5. Hanson MB, Van Nagell JR, Powell DE, et. al.: The prognostic significance of lymph-vascular space invasion in stage I endometrial cancer. Cancer 55:1753–1757, 1985.
5. Geisinger KR, Homesley HD, Morgan TM et al.: Endometrial carcinoma: a multiparameter clinicopathologic analysis including the DNA profile and the sex steroid status. Cancer 58:1518–1525, 1986.
7. Iversen OE: Flow cytometric deoxyribonucleic acid index: A prog-

nostic factor in endometrial carcinoma. Am J Obstet Gynecol 155:770–776, 1986.
8. Britton, LC, Wilson TO, Gaffey TA, et al.: Flow cytometric DNA analysis of stage I endometrial carcinoma. Gynecol Oncol 34:317–322, 1989.
9. Britton LC, Wilson TO, Gaffey TA, et. al.: DNA ploidy in endometrial carcinoma: Major objective prognostic factor. Mayo Clin Proc 65:643–650, 1990.
10. Newbury R. Schuerch C, Goodspeed N, et. al.: DNA content as a prognostic factor in endometrial carcinoma. Obstet Gynecol 76:251–257, 1990.
11. Moberger B, Auer G, Forsslund G, Moberger G: The prognostic significance of DNA measurements in endometrial carcinoma. Cytometry 5:430–436, 1984.
12. Friedlander MF. Hedley DW, Taylor IW: Clinical and biological significance of aneuploidy in human tumors. J Clin Pathol 37:961–974, 1984.
13. Sorbe B, Risberg B, Thornthwaite J: Nuclear morphometry and DNA flow cytometry as prognostic methods for endometrial carcinoma. Int J Gynecol Cancer 4:94–100, 1994.
14. Atkin NB: Prognostic significance of ploidy level in human tumors. I Carcinoma of the uterus. J Natl Cancer Inst 56:909–910, 1976.
15. Russack V: Image Cytometry: Current Applications and future trends. Crit Rev Clin Labor Sci 31:1–34, 1994.
16. De Palo G, Kenda R, Andreola S et. al: Endometrial carcinoma: Stage I, A retrospective analysis of 262 patients. Obstet Gynecol 60:225–231, 1982.
17. Boronow RC, Morrow CP, Creasman WT et. al.: Surgical staging in endometrial cancer: Clinicopathological finding of a prospective study. Obstet Gynecol 63:825–832, 1984.
18. Christopherson WM, Connelly PJ, Alberhasky RC: Carcinoma of the endometrium: V. An analysis of prognosticators in patients with favorable subtypes and stage I disease. Cancer 51:1705–1709, 1983.
19. Lindahl B, Ranstam J, Willen R: Five year survival rate in endometrial carcinoma stages I–II: Influence of degree of tumour differentiation, age, myometrial invasion and DNA content. Br J Obstet Gynaecol 101:621–625, 1994.
20. Harouny VR, Sutton GP, Clark SA, et. al.: The importance of peritoneal cytology in endometrial carcinoma. Obstet Gynecol 72:394–398, 1988.
21. Vecek N, Marinovic T, Ivic J, et. al.: Prognostic impact of peritoneal cytology in patients with endometrial carcinoma. Eur J Gynaecol Oncol 14:380–385, 1993.